

PATENT  
Docket No. 381092000700

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*Karen LePari*  
Karen LePari

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

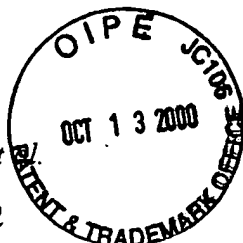
In the application of:

Terry P. Snutch, et al.

Serial No.: 09/030,482

Filing Date: 25 February 1998

For: NOVEL HUMAN CALCIUM  
CHANNELS AND RELATED PROBES,  
CELL LINES AND METHODS



Examiner: Nirmal S. Basi

Group Art Unit: 1646

DECLARATION OF TERRANCE P. SNUTCH

Assistant Commissioner for Patents  
Washington, D.C. 20231

Dear Sir:

I, Terrance P. Snutch, declare as follows:

1. I am a co-inventor of the subject matter claimed in the above-referenced application and have been practicing in the field of molecular biology for over twenty years. I am very familiar with cloning techniques and hybridization conditions.
2. In my opinion, the designation of hybridization conditions as those of medium stringency would convey to those of skill in the art that nucleotide sequences encoding closely related members of the same family of molecules would hybridize, but those outside this closely related family would not. In the present case, it would be understood that members of the family

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represented by the newly discovered ion channel family described herein would hybridize to the disclosed nucleotide sequences, but nucleotide sequences encoding ion channels presently known in the art would not.

3. There are many examples in the literature whereby hybridization stringency is referred to as high, medium or low based upon temperature and salt conditions (*e.g.* Snutch, T.P., Heschl, M.F.P. and Baillie, D.L. (1988). The *Caenorhabditis elegans* hsp70 gene family: a molecular genetic characterization. *Gene* 64:241-255; Yu, A.S.L., Hebert, S.C., Brenner, B.M. and Lytton, J. (1992). Molecular characterization and nephron distribution of a family of transcripts encoding the pore-forming subunit of  $\text{Ca}^{2+}$  channels in the kidney. *Proc. Natl. Acad. Sci. USA* 89:10494-10498).

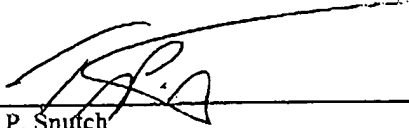
4. Medium stringency hybridization to colony or plaque lifts fixed on nitrocellulose or nylon membranes is typically performed 62°C to 65°C in the presence of probe in a solution containing 5 times Denhardt's, 0.3% SDS and 5 X SSPE. Non-specific carrier DNA such as denatured salmon sperm DNA (100 to 200 ug/ml) and a hybridization accelerator such as dextran sulfate (10%) may also be included in the hybridization buffer. Alternatively, medium stringency hybridization may be performed at 42°C in a solution containing 50% formamide, 5 times Denhardt's, 0.3% SDS and 5 X SSPE. The exact concentration of SDS and SSPE can vary and there are reports of SDS utilized from 0.2% to 0.7% and SSPE from 5 X to 6 X or the alternative buffer SSC from 5 X to 6 X. After hybridization the hybridization solution is removed and membranes are washed several times in a solution typically containing 0.1 % to 0.3% SDS and 2 X SSPE to 0.2 X SSPE. The temperature of medium stringency washing typically can vary from 55°C to 65°C.

5. Detailed information concerning the relevant considerations to be taken into account for determining hybridization stringency can be found in: Basic Methods in Molecular Biology (1986) Edited by Davis, L.G., Dibner, M.D. and Battery, J.F. Elsevier Science

Publishing Co., New York, and in DNA Probes (1989) Edited by Keller, G.H and Manak, M.M. Stockton Press, New York.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Executed at Vancouver, BC on 10 October 2000.



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Terrance P. Snutch